227 Effective association of tea tree essential oil with conventional antibiotics to control *Pseudomonas aeruginosa* biofilms

Filipa Lobo Coelho, Susana Patrícia Lopes, Maria Olívia Pereira
IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710–057 Braga, Portugal.

Pseudomonas aeruginosa is an important cause of acute nosocomial and biofilm-associated infections that are often difficult to eradicate. The increase of antimicrobial resistance, especially in sessile microorganisms, has promoted the search for new alternatives, as natural products, to control biofilm infections. Essential oils are natural, volatile, and complex compounds produced by aromatic plants as secondary metabolites[1]. Tea tree essential oil (TTO) is obtained from Melaleuca alternifolia, and it has been reported that exhibits a broad-spectrum antimicrobial activity that can be explored as an effective alternative to control biofilms[2,3]. This study aimed to assess the antibacterial activity of TTO associated with ciprofloxacin (CIP), a conventional antibiotic, against pre-formed P. aeruginosa biofilms, using two different application strategies: aggression of biofilms simultaneously with TTO+CIP, and aggression of biofilms firstly with TTO and then with CIP. Biofilms were characterized, before and after treatments, by total biomass, through crystal violet, and number of cultivable bacterial cells (log CFU/cm2). The association of the two compounds emerged after inspection of its individual antimicrobial activities that showed that biomass of P. aeruginosa biofilms was increasingly reduced when biofilms were treated with TTO, though it not significantly affected the viability of cells. On the other hand, CIP showed no considerably interference in biofilm control, since the reduction of biofilm biomass and number of viable cells was almost nonexistent, even for the highest concentration tested (80 μg ml-1). Results related with the combination of TTO and CIP showed that both strategies experienced were very efficient, as they impaired significantly biofilm formation. It was observed a considerable decrease of biofilm biomass (> 70 %) and number of cells (>3 log of reduction) even for 1.25 μg ml-1 of CIP, when biofilms were aggressed with the TTO followed by CIP. The synergistic effect of TTO combined with CIP also resulted in a high decrease of biomass and a completely eradication of viable cells for 10 μg ml-1 of CIP. These data highlighted the relevance of using natural products, as essential oils, to enhance conventional antibiotics activity, reducing the necessary concentration to treat an infection, and consequently reducing its toxicity and side effects. Therefore, this data provides further evidence that TTO-ciprofloxacin combinations may be a useful therapeutic option in the treatment of P. aeruginosa biofilm associated infections.

Acknowledments: The financial support from IBB-CEB, FCT and European Community fund FEDER, trough Program COMPETE, in the ambit of the project PTDC/SAUSAP/113196/2009/FCOMP-01-0124-FEDER-016012 is gratefully acknowledged.

References: 1 - Bakkali F, Averbeck S, Averbeck D, Idaomar M, Biological effects of essential oils - A review. Food and Chemical Toxicology, 2008. 46: p. 446-475. 2 - Budzynska A, Wieckowska-Szakiel M, Sadowska B, Kalemba D, Rózalska B, Antibiofilm Activity of Selected Plant Essential Oils and their Major Components. Polish Journal of Microbiology, 2011. 60: p. 35-41. 3 - Kwiecinski J, Eick S, Wójcik K, Effects of tea tree (Melaleuca alternifolia) oil on *Staphylococcus aureus* in biofilms and stationary growth phase. International Journal of Antimicrobial Agents, 2009. 33: p. 343-347.

Keywords: Biofilms, Tea tree essential oil, Conventional antibiotics, *Pseudomonas aeruginosa*.